

Congressional Justification

National Institute of Dental and Craniofacial Research

Authorizing Legislation: Section 301 of the Public Health Service Act, as amended.
Reauthorizing legislation will be submitted.

Budget Authority:

	2001 <u>Actual</u>	2002 <u>Appropriation</u>	2002 Current <u>Estimate</u>	2003 <u>Estimate</u>	Increase or <u>Decrease</u>
Current Law BA	\$304,606,000	\$343,327,000	\$343,149,000	\$372,167,000	\$29,018,000
Accrued Costs	1,999,000	2,154,000	2,154,000	2,152,000	(2,000)
Proposed Law BA	306,605,000	345,481,000	345,303,000	374,319,000	29,016,000
FTE	290	300	300	299	(1)

This document provides justification for the FY 2003 activities of the National Institute of Dental and Craniofacial Research (NIDCR), including HIV/AIDS activities. A more detailed description of NIH-wide Fiscal Year 2003 HIV/AIDS activities can be found in the NIH section entitled "Office of AIDS Research (OAR)."

The President's appropriations request of \$374,319,000 for this account includes current law adjusted by assuming Congressional action on the proposed Managerial Flexibility Act of 2001.

Introduction

Over the past 50 years, our nation's investment in dental and craniofacial research has yielded tremendous advances in American public health. Some of the often-cited examples include a sharp reduction in the once rampant rate of dental caries and tooth loss, improved care of all aspects of gum (periodontal) diseases, and the effective management of oral pain.

However, NIDCR's mission to improve the nation's oral health remains far from finished. One reason is the fact that many of the nation's oral health advances have yet to fully benefit our underserved populations. Specifically, NIDCR plans to push forward and reduce the higher

incidence of oral cancer, gum disease, and tooth decay among the underprivileged in our society.

The NIDCR remains committed to forwarding this effort and pursuing it to its rightful conclusion. As a first step, NIDCR, in collaboration with the National Center on Minority Health and Health Disparities (NCMHD), has funded five Centers for Research to Reduce Oral Health Disparities at Boston University, New York University, the University of California at San Francisco, the University of Michigan, and the University of Washington. Another study has also been funded that is examining the underlying causes of oral health disparities in rural West Virginia. This year, NIDCR will try to fill additional gaps by seeking out investigations studying underserved and special populations not addressed by the newly funded Centers and study.

The NIDCR leadership also recognizes that scientists today truly stand on the threshold of an unprecedented “Golden Age” in biology. The recent completion of the Human Genome Project, in tandem with the emergence of more powerful research technologies in the laboratory, are allowing scientists to catalogue with encyclopedic comprehensiveness the actual genes, proteins, and protein networks that power our cells. Such studies, an impossibility just a few years ago, have opened a valuable window into the genetic programs of some of life’s most intriguing developmental processes, including the formation of the human face.

Just as importantly, this vantage point provides a means for scientists to pinpoint the very molecules that trigger some of our most common oral health problems. These discoveries will lay the foundation for a more systematic exploration of the complex interplay of genetics, environment, and behavior that leads to disease. They also will yield valuable biological information, which can then be leveraged to design more accurate diagnostics and more targeted therapies for complex and poorly understood oral problems such as temporomandibular joint disorders, cleft lip and palate and Sjögren's syndrome.

This report will outline some of the many exciting NIDCR-supported advances during the past fiscal year. It also highlights specific, potentially high-yield scientific opportunities that now exist to improve the nation’s oral health. These opportunities represent not only hope for millions of Americans today, but improved health and quality of life for generations to come.

Relieving Acute and Chronic Pain

Different people can experience the same pain stimulus differently. For this reason, the study of pain can be a lot like trying to analyze multiple moving targets at once. Among the variables involved in the pain process are: age, immune function, endocrine and neural activity, genetics, stress, psychological state, gender, and even cultural background.

However, despite the inherent complexity of their work, NIDCR-supported scientists continue to make progress in understanding the dynamics of pain and how to effectively control it in dental care and for pain sufferers in general. Here are some recent examples:

Science Advance: The Brain's Painkiller System in Action

NIDCR grantees used positron emission tomography (PET) to image the brain's chemical activity while human volunteers received a stimulus mimicking the chronic pain of a temporomandibular joint disorder (TMD). This marked the first time ever that scientists had analyzed sustained pain by simultaneously monitoring brain scans of a key neurochemical system and recording the self-reported pain ratings of human participants.

The scientists found that while experiencing pain in the jaw muscles for 20 minutes, the volunteers had a surge in the release of natural opioids, part of the brain's painkilling system, and a concomitant drop in pain and pain-related emotions. But most significantly, the researchers discovered major variation among volunteers in both baseline and pain-induced levels of naturally occurring opioids. Baseline levels were measured by examining the participants while they received a placebo. Interestingly, when comparing placebo and pain-inducing conditions, the activation of the anti-pain response was dramatic in some volunteers, while in others it was much less pronounced. Those who had the greatest change tended to report the lowest experience of pain, both in its sensory and emotional aspects.

This study provides insights into the body's natural painkiller system and the reasons why each of us experiences pain differently. The results also show how brain chemistry regulates sensory and emotional experiences. The findings may help researchers better understand chronic pain and find more effective ways to relieve it.

New Initiative: Temporomandibular Joint Disorders

Temporomandibular joint disorders are conditions affecting the joint that connects the lower jaw (the mandible) to the skull and the surrounding muscles that are used to chew and open the mouth. An estimated 5 to 12 percent of Americans report having pain associated with the temporomandibular joint. Studies suggest that TMD may be as much as two times more common in women than men.

Despite its prevalence, TMD remains poorly defined and inadequately understood. However, the opportunity now exists to apply recent advances in genetics, molecular biology, and biotechnology to TMD and expand the breadth of scientific investigation into these disorders. To seize this opportunity, NIDCR will launch a new initiative to define the molecular and physiological basis of these conditions. The initiative will be multidisciplinary and multifaceted, including:

Establishment of the first TMD patient registry - The registry will be crucial in tracking the incidence and natural history of these conditions. It also will allow NIDCR to launch more rapidly the necessary clinical trials to evaluate promising diagnostic and therapeutic leads as they

emerge.

Identify biomarkers - NIDCR will make a concerted effort to identify biomarkers--genes, proteins, or even protein networks--that are adversely affected by TMD and which can serve as a telltale sign of a developing or progressing condition. By developing a battery of sensitive and highly specific diagnostic and prognostic biomarkers, critical molecular information will be available to more accurately diagnose and treat TMD, a long-held hope of many Americans affected by these conditions.

Creation of animal models that closely mimic TMD - With the arrival of more powerful research tools that generate vast amounts of biological information, new hypotheses can be generated faster than ever. To test these hypotheses, NIDCR will continue to support the development of laboratory animals such as mice, rabbits and pigs that more accurately mimic the symptoms and pathology of TMD. Without them, the pace of this initiative will be slowed.

The Genetics of Craniofacial Diseases

The physical complexity of the human head and face has captured the imagination of artists since the beginning of time. However, this exquisite complexity sometimes can be problematic for clinicians who must treat injuries, diseases and genetic defects of the craniofacial region. The NIDCR already has made major strides in unraveling the molecular dynamics of craniofacial development and the tools are now at hand for even greater advances in the future.

Science Advance: Mouse Model Sheds Light on Hereditary Dental Defect

Amelogenesis imperfecta (AI) is a hereditary disorder in which teeth have abnormal enamel. One of the likely culprits in causing AI is the amelogenin proteins, which comprise about 90 percent of the lattice-like extracellular matrix. Scientists believe amelogenins play an important role in forming enamel in developing teeth, although their precise functions are not well understood.

To learn more about these proteins, NIDCR researchers have created a mouse model that mimics AI. The researchers produced the mice by deleting, or knocking out, the amelogenin gene. These bioengineered mice developed abnormal teeth with chalky white discoloration as early as two weeks of age, as well as an abnormally thin layer of tooth enamel. Through scanning electron microscopic analysis, the researchers found that the enamel lacked the "prism" pattern that is the hallmark of the normal, highly ordered, enamel crystal. These findings revealed that the amelogenins are apparently not required for enamel to form, but are necessary for the organization of the distinctive crystal pattern and the regulation of enamel thickness. This mouse model will continue to be useful for understanding the functions of amelogenin proteins during enamel formation and for developing therapeutic approaches for treating amelogenesis imperfecta.

Science Advance: Studies of Apert Syndrome

Apert syndrome is a genetic disorder characterized by the premature closing of the skull's bony plates and severe webbing of the hands and feet. It occurs in approximately one in 160,000 to 200,000 live births. There are two major forms of this disorder, one which frequently involves cleft palate, and the other in which patients exhibit more severe webbing of toes and fingers. Like many human skeletal disorders, Apert syndrome is caused by mutations in the gene that encodes the cell-surface receptor that binds fibroblast growth factor. Using X-ray crystallographic analysis, NIDCR grantees compared the three dimensional structure and shape of normal and mutated fibroblast growth factor receptors. They found that both mutations associated with Apert syndrome resulted in stronger binding of the receptor to the fibroblast growth factor. Increased binding attachment upsets the balance between receptor activation and inactivation, leading to inappropriate signaling. Based on the structural analyses, the researchers propose that changes in the binding affinity and specificity produced by the two fibroblast growth factor mutations may account for differences in the physical anomalies seen in the two subgroups of Apert syndrome patients.

This report is believed to be the first to describe the crystal structures of mutant receptors implicated in human disease bound to their partner "ligands". The study demonstrates the usefulness of structural biology to explain the molecular basis of a genetic disorder. In addition to providing a structural basis for understanding both forms of Apert syndrome, these crystal structures also establish a framework for engineering artificial fibroblast growth factors for use in treating Apert syndrome as well as other growth regulatory disorders such as cancer.

Using Bioengineering Approaches to Stimulate Growth and Healing

Biomimetics and bioengineering take their clues from nature to discover novel approaches to repair and replace bodily tissues. Approaches that imitate the body's own healing and regenerative processes are especially promising for oral and craniofacial conditions. One example is Sjögren's syndrome, an autoimmune disease that affects over one million Americans, predominantly women. The condition leads to irreversible damage of salivary gland tissue, leading to a loss of saliva production and an increased risk of developing dental caries and other oral infections. For people with Sjögren's syndrome, their quality of life is compromised by the ensuing "dry mouth," which causes difficulty in chewing, swallowing and speaking. Since no effective therapy has yet been developed, NIDCR has placed a high priority on the study of Sjögren's disease.

To help people with Sjögren's syndrome, NIDCR researchers are using a biomimetic approach to develop an artificial salivary gland. The Institute also has initiated efforts to construct a registry of Sjögren's syndrome patients to enable researchers and clinicians to have access to the broadest possible pool of data on this complex disease.

Science Advance: New Evidence of Circulating Skeletal Stem Cells

People have two types of (adult) stem cells: hematopoietic, which form the blood elements, and stromal, which give rise to all major skeletal tissues, including bone and cartilage. While hematopoietic stem cells circulate without sticking to tissues, that is not the case with stromal cells. They are adherent, non-circulating, and are capable of forming collagen (termed fibroblastic), the major protein that makes up skeletal tissues. Scientists had previously speculated that blood may also contain cells that can become adherent and display fibroblastic properties, but the existing data were not definitive.

NIDCR researchers have now established the existence of adherent, fibroblastic-like cells in the blood of four types of mammals. These circulating skeletal stem cells resemble, but are not identical to, stromal stem cells. The researchers were able to isolate these cells from the blood of adult mice, rabbits, guinea pigs and humans. When transplanted into mice the cells formed bone, giving the first evidence that adherent, fibroblast-like cells circulating in blood can form bone.

Further research may establish the ability of these circulating skeletal stem cells to give rise to other types of tissues. Additionally, the evidence of their existence offers researchers an accessible source of adult stem cells for potential therapeutic use.

Science Advance: Potential Use of Human Dental Pulp Stem Cells in Tissue Engineering

Beneath the enamel of the tooth is the dentin, a hard structure that surrounds the soft dental pulp. Dentin is formed by odontoblasts, specialized cells believed to arise from stem cells in dental pulp. Until now, no one had ever isolated the precursor cells in adult dental pulp that give rise to odontoblasts.

Using methodology developed to isolate and characterize bone marrow stem cells, NIDCR scientists have isolated stem cells from the pulp of adult human teeth and grown them in the laboratory. They transplanted the cells into mice, where they generated a dentin-like structure. The amount of tissue formed in the transplants far exceeded the amount an individual would normally make during a lifetime. This research demonstrates that adult dental pulp contains cells that are highly proliferative, and capable of regenerating tissue-- properties that effectively define them as stem cells.

This study suggests that it may be possible to use adult dental pulp stem cells in tissue engineering. A single tooth could possibly yield enough stem cells to repair damaged dentin in a number of teeth, potentially revolutionizing the treatment of dental decay and the delivery of root canal therapy. In the future, researchers may be able to use the stem cell technology to fabricate new teeth to replace those lost through injury or diseases such as amelogenesis imperfecta.

Ongoing Area of Emphasis: Restoring Health to Orofacial Tissues and Organs Using Biomimetic, Tissue Engineering and Stem Cell Approaches

Biomimetics and tissue engineering are two relatively new scientific fields. Biomimetics studies the process of how nature designs and produces its various tissues, such as skin, bone, and tendon. Based on the principles of biomimetics, tissue engineers fabricate unique molecules and materials that promote the growth of new tissues that are lost due to disease, trauma, or congenital defects.

One area of great interest within both disciplines is stem cell research. This interest results from the fact that stem cells are capable of generating many specialized cell types. The so-called plasticity of stem cells provides opportunities to develop unique strategies for the repair and regeneration of orofacial structures that are faulty or damaged as a result of congenital disorders, disease, or injury.

NIDCR continues to fund research that will lead to the development of safe and effective stem cell-based treatments, including research to understand the signals, or cues, that regulate capacity of stem cells for self-renewal, determination of cell-type, and maintenance of orofacial tissue. The goal of this initiative is to foster research on approved human and mouse embryonic and adult stem cell biology that could help define in greater detail the complex events that occur during normal oral, dental, and craniofacial development and disease. Specifically, this initiative will focus on restoring salivary gland function; repairing and regenerating teeth, gums, and the bones that support these tissues; and developing diagnostic and treatment strategies for temporomandibular joint repair and restoration. Understanding the mechanisms of stem cell renewal not only shows great potential to revolutionize the prevention and treatment of many complex oral and craniofacial disorders, but it also could provide insight into events such as tumorigenesis, the mechanism by which a cancer stem cell recreates.

Detecting, Preventing and Treating Head and Neck Cancer

During the past decade, science has made enormous progress in understanding the genetic, molecular, and even environmental causes of cancer. Whereas a decade ago researchers celebrated the discovery of individual “cancer” genes, they today have assembled vast databases of cancer-associated genes and proteins, possible clues for future molecularly targeted treatments. Scientists once searched for a single “magic bullet” to cure cancer. Today they speak of attacking tumors on multiple fronts, ranging from standard chemotherapy drugs to choking off their blood supply or targeting immune cells to deliver deadly poisons. This newfound excitement in cancer research extends to the study of head and neck cancers. Once poorly understood, today head and neck cancers are among the most well characterized tumors in cancer research. With a continued investment in this area, further advances will undoubtedly accrue.

Science Advance: Early Detection of Head and Neck Squamous Cell Carcinoma

Oral, pharyngeal, or laryngeal cancer is diagnosed in an estimated 41,000 Americans each year. Patients whose cancers are detected and treated early have a much better chance of survival and suffer less treatment-related damage than patients whose disease is diagnosed late.

Unfortunately, many head and neck cancers are not detected early, resulting in a poor prognosis.

The reasons for late detection range from failure by patients to recognize early symptoms or to seek medical care promptly, to difficulty in detecting some tumors during clinical examination. A reliable, noninvasive test to detect head and neck cancer early, perhaps even before it is clinically detectable, would offer a means of reducing morbidity and mortality using current treatment options.

NIDCR-supported scientists compared cancer-specific changes in tumor tissue and oral cheek cell DNA using a technique called microsatellite analysis. Tumor tissue was analyzed from 44 patients with head and neck cancer and in cells collected by oral rinsing and swabbing from the cancer patients and from 43 healthy control subjects. They found DNA changes signaling cancer in 38 of the tumors, and found matching genetic alterations, or cancer markers, in the oral samples from 35 of the 38 patients whose tumors contained markers. No cancer-specific genetic changes were found in oral cells collected from the healthy subjects. Cancer markers were detected in both lymph node metastases and oral cells from three patients with unknown primary tumor sites, indicating that microsatellite analysis of saliva can reveal cancer not detectable by oral examination.

The study provides proof of principle for using microsatellite analysis of cells in saliva to detect head and neck cancer--the first step in developing a reliable, noninvasive screening test for these cancers. If the test is validated and refined, it could provide clinicians with a tool for screening at-risk persons for early-stage head and neck cancer and for monitoring patients after cancer treatment.

Science Advance: Targeted Drug Delivery to Head and Neck Tumors

Chemotherapy is used for organ preservation, management of recurrent disease, and in combination with radiotherapy in the treatment of head and neck tumors. But since chemotherapy harms healthy cells as well as tumor cells, it produces side effects that sometimes limit the amount of drug that can be given. To avoid untoward effects, scientists have attempted to create molecular sentinels that can distinguish tumor cells from normal cells as a means to deliver anti-cancer agents directly to diseased cells. NIDCR researchers have successfully isolated a protein fragment (peptide HN-1) that meets several criteria for targeted drug delivery into solid tumors. The study showed that the peptide binds to the cancer cells but not to adjacent noncancerous tissues. Additionally, HN-1 can be detected both in central portions of the tumors as well as in peripheral tumor cells suggesting that it is able to penetrate the tumor tissue. The study also showed that HN-1 can be internalized by human head and neck squamous cell

carcinoma cells and therefore would be capable of transferring drugs across the cell membranes, a critical requirement for drug delivery. Moreover, the peptide localizes to tumors (in mice) when injected into the tail vein, indicating that it would be an effective carrier for an anti-cancer agent when administered intravenously. Interestingly, this peptide appears to be specific for head and neck squamous cell carcinomas.

These studies establish the potential use of this tumor-specific peptide for targeted drug delivery to head and neck tumors. Specifically, use of this peptide attached to a chemotherapeutic agent may provide the maximally effective dose of a drug to destroy tumors, without producing harmful side effects to other cells.

HIV Infection and HIV-Related Opportunistic Infections

The fluids that coat the mucosal surfaces of the body, including the mouth, lung linings, digestive tract, and urogenital tract protect these delicate tissues from the harsh external environment. Among the components of this protective fluid are a set of unique antibodies (secretory antibodies) that are particularly resistant to breakdown by bacterial and viral products. Scientists are now attempting to exploit the secretory immune system as a means of augmenting defense against a whole spectrum of infectious agents, including HIV, that gain entry into the body via the mucosa.

New Initiative: Oral Mucosal Vaccination Against HIV Infection and HIV-Related Opportunistic Infections

Recent research suggests that vaccines targeted to mucosal tissues may elicit significant mucosal and systemic responses. However, antigens (designed to elicit the formation of the needed antibodies) delivered by mucosal routes may become degraded due to the many bacteria and viruses that line the mucosal surface or they simply may elicit only a weak immune response. Different antigen preparations, including those using immunomodulatory molecules and adjuvants, and various delivery systems may enhance the protective nature of the mucosal immune response.

This initiative will support the development of new strategies for vaccination against HIV and HIV-associated infections by oral and nasopharyngeal routes. Scientists will capitalize on the exciting advances in mucosal immunology to develop ways to immunologically block HIV infection and its spread.

Understanding and Preventing Oral Infections

Although advances in prevention have lowered the incidence of oral infections such as caries and periodontal disease, these conditions still cause significant pain and suffering throughout the

United States. Further success in combating oral infections has remained elusive because so many factors, including infectious agents, behavior, genetic susceptibility, and overall health status all play a role in the etiology of these diseases. However, new developments in understanding these conditions and the factors that promote or inhibit them offer promise in further reducing oral infections.

Science Advance: Natural Product May Inhibit Dental Caries

Propolis, a resinous product collected from beehives, has been used for thousands of years in folk medicine to treat wounds, ulcers, and diseases such as mouth and throat infections. Bees gather propolis from the buds of various poplar and conifer trees, mix it with beeswax and salivary secretions, and then use the substance to strengthen and protect their hive from germs and foreign invaders. Propolis also has antibacterial and antifungal effects that protect the bee colony against disease.

NIDCR-supported researchers have found that propolis significantly reduces dental plaque formation. Propolis is a potent inhibitor of the molecular machines called glucosyltransferases (Gtf), which synthesize sticky glucans from table sugar (sucrose). Glucans promote the binding of harmful bacteria to teeth, which is a critical step in the development of dental decay. Interestingly, the investigators discovered that the effectiveness of propolis depended on the geographic area from which it was collected.

The current study demonstrates that propolis is a potent inhibitor of one important variety of Gtf. This level of inhibition has not been observed before. Further characterization of the structure and function of the active component of propolis may lead to a new anti-caries product.

New Initiative: The Role Of Microbial Biofilms in the Pathogenesis of Oral Disease

A subset of microorganisms organize themselves in “communities” at the interface between the external environment and the host mucosal or tooth surface. Microorganisms that grow in these communities or biofilms may have markedly different properties than the same organisms growing in a liquid environment. Understanding the processes responsible for the formation, integrity and protective nature of microbial biofilms will allow us to discover novel and sophisticated methods of treating various microbial diseases. Oral biofilms have diverse microbial populations and serve as reservoirs for the bacteria that cause caries, periodontal diseases and opportunistic localized or systemic infections. The objective of this initiative is to stimulate fundamental research that will lead to improved clinical control of oral biofilms.

Both bacterial products and host secretions add to the scaffolding of the biofilm. Structural changes alter the strength of the biomass and make the biofilm more or less susceptible to external agents such as metal ions, antibiotics or bacteriostatic agents. Improved understanding of biofilms structure can lead to breakthroughs in treatment of oral diseases. Bacteria within oral

biofilms undergo simple synergistic associations such as sharing nutrients and making pH adjustments. Such interactions are central to the growth and survival of the biofilm. Recent evidence suggests that the bacteria “talk” to one another through the release of chemicals that can alter gene expression in the recipient bacteria. Gaining a comprehensive understanding of these communications is expected to inform new approaches to developing diagnostics and treatments for oral diseases.

Story of Discovery

Salivary Glands: Potential Target Site for Gene Therapies

For millions of people who suffer from diseases such as diabetes, growth hormone deficiency or hemophilias--diseases caused by a deficiency in a single protein--gene therapy may someday offer an attractive alternative to available treatments. Current therapies for these disorders are invasive. Primarily administered by injection, the treatments are costly and often dependent upon patient self-monitoring and compliance. Furthermore, they are not cures. Gene therapy offers the potential to correct the underlying disorder in these patients by actually replacing the missing protein.

NIDCR researchers are studying the possibility of using gene transfer in salivary glands to produce missing hormonal proteins. Gene transfer to salivary glands is an exciting prospect because it is noninvasive. The ducts of major salivary glands exit into the mouth and can be readily accessed without any surgical procedure. Furthermore, salivary glands are a natural protein-producing site, making them an appealing choice for the delivery of gene products. Although salivary glands are exocrine glands--they secrete outwardly through ducts--scientists have speculated for years that they could also act as endocrine glands, which secrete internally into the bloodstream.

NIDCR scientists began gene transfer studies about a decade ago. Their first important step was to demonstrate that using gene transfer technology, salivary glands could indeed be coaxed into producing “foreign” proteins and secreting them into the bloodstream. Delivery of a therapeutic gene requires the use of a vector, or vehicle, to carry it. Using an adenovirus (cold virus) as the vehicle, the researchers inserted a gene that encodes for alpha-1-antitrypsin--a serum protein normally made and secreted by the liver--into the adenovirus. They then administered the adenovirus into the salivary glands of rats with the hope that the glands would begin to produce the protein. Results of the study clearly showed that salivary glands could be made to produce and secrete something other than their own natural product. The research provided the first direct evidence that salivary glands could secrete a transgenic protein, alpha-1-antitrypsin, into the bloodstream, as confirmed by measurement of the protein levels in blood. In every rat examined in the study, levels of alpha-1-antitrypsin were higher in the veins coming out of the salivary gland than in those going in, clearly showing that the protein was being secreted into the bloodstream.

Next, the investigators needed to determine if transgenic proteins secreted into the bloodstream from salivary glands were biologically active. That is, did the transgenic proteins have the ability to perform their intended function? NIDCR researchers used an adenoviral vector encoding human growth hormone (hGH) to find out. After delivering the hGH gene to rat submandibular salivary glands, they analyzed serum from the treated animals. They found several chemical changes indicating that the hGH protein was functioning as intended. Furthermore, the animals’ hGH blood levels were three times those required physiologically, proving that the salivary gland can produce the transgenic protein in abundance.

But how exactly were the transgenic proteins produced by the salivary glands getting into the bloodstream? For salivary glands to be useful in gene therapy, transgenic proteins must be efficiently directed into the bloodstream

for systemic use. Proteins are normally secreted from cells via two general pathways, constitutive and regulated paths. While many cells have constitutive pathways, a regulated pathway requires a sophisticated cell. NIDCR investigators knew that salivary gland cells secrete salivary proteins through a regulated pathway. They suspected that the cells might also be using a constitutive pathway to secrete the transgenic proteins into the bloodstream. This hypothesis was tested using three proteins, each inserted into rat salivary glands using an adenoviral vector. The investigators then measured the amount of each transgene product in saliva and blood. While two of the proteins were secreted primarily into saliva, the third was secreted into the bloodstream. The study revealed that salivary glands are able to sort the transgenic proteins into the two distinct pathways by recognizing sorting signals presumably encoded in the proteins. Understanding these signals and how to manipulate them would help in the efficient delivery of these therapeutic proteins to specific sites.

However, the studies exposed a major concern--that some of the proteins, including hGH, were predominantly secreted into saliva where they are not biologically useful. NIDCR researchers tested their theory that a common manipulation used in cell biology sorting experiments might be able to re-direct protein secretion from salivary glands into the bloodstream. The researchers injected the rats with the alkalinizing agent hydroxychloroquine (HCQ), an FDA-approved drug, prior to delivering the adenovirus into the rats' salivary glands. HCQ changes the pH level of intracellular vesicles, which are the "cargo ships" carrying secretory proteins along their route within the cell; when this happens, their internal compasses malfunction. With their pH level off balance, the vesicles become lost and confused, and are more likely to exit through the back of the cell into the bloodstream (the endocrine path) rather than through the front (the exocrine path). Results of the study indicated that HCQ markedly enhanced, by more than 10-fold, the proportion of transgenic proteins secreted from salivary glands into the bloodstream. As an added benefit, investigators learned that the addition of HCQ allows them to use significantly less virus to transfer the gene into the glands. This may someday yield a safer procedure for clinical use in humans.

NIDCR's decade-long pursuit of producing biologically active substances in human salivary glands now appears to be within reach. Animal studies have revealed how to deliver hormonal proteins from salivary glands into the bloodstream at useful levels, without using high doses of a viral vector. Researchers now will try to determine if this gene transfer technology--including the use of HCQ--will enhance secretion of transgenic proteins in humans. Further studies also will be in progress to find a way to regulate secretion of the hormones such that they are made only as needed and in the amounts required. Clinical testing is the next important step in this journey.

Budget Policy

The Fiscal Year 2003 budget request for the NIDCR is \$374,319,000 including AIDS, an increase of \$29,016,000 and 8.4 percent over the FY 2002 level.

A five year history of FTEs and Funding Levels for NIDCR are shown in the graphs below. Note that Fiscal Years 2000 and 1999 are not comparable for the Managerial Flexibility Act of 2001 legislative proposal.